The role of soil organic matter on denitrification potential in newly created wetlands

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Introduction

Denitrification is a process occurring in anaerobic environments in which microbes convert nitrate-nitrogen (NO_3^-) to gaseous nitrous oxide (N_2O) and molecular nitrogen (N_2). In wetlands, a narrow oxidized layer, present just above the anaerobic wetland soils, causes a combination of reactions that can result in significant nitrogen losses to the atmosphere (Mitsch and Gosselink, 1993). Due to the fact that nitrous oxide is a "greenhouse gas," measuring its emission from denitrification in natural ecosystems like wetlands could be extremely useful in comparisons to managed systems that employ nitrogen fertilizers (Goodroad and Keeney, 1984). Denitrification is a major contributor to the function of wetlands in water quality. The process removes nitrate, a drinking water pollutant and the major cause of coastal eutrophication. Denitrification may keep nitrate from moving from runoff and groundwater to surface waters, as well (Gilliam, 1994). In constructed wetlands, the nitrogen retention can be approximately 50% with inflow rates of less than 100 g N m^{-2} yr^{-1}; most of this retention can be attributed to denitrification (Mitsch and Gosselink, 1993).

Several studies have been conducted that link the availability of nitrogen and carbon with soil denitrification rates. Bradley et al. (1992) studied anaerobic groundwater sediments and found that although nitrate concentration differences accounted for a substantial amount of potential denitrification rate variation—approximately 34%—the more significant limiting factor was the organic carbon content of the sediments, which accounted for about 80% of the variation. The effects of soil water, carbon availability and nitrate concentration in four benchmark soils were investigated by Weier et al. (1993). The additions of glucose-carbon and nitrate produced variations in total denitrification in all four soils. However, because microbial respiration increased at higher concentrations of carbon, regardless of the concentration of nitrogen in the soil, carbon was found to be the more significant limiting factor. Microbes need a source of energy before denitrification with any amount of nitrate can occur. Groffman and Hanson (1997) conducted a study of different substrates in and around a red maple (*Acer rubrum*) wetland site. Their results indicated that nutrient-rich soils supported higher denitrification rates than soils lacking sufficient amounts of nutrients like carbon and nitrogen. Nutrient-poor soils support vegetation that require fewer nutrients, and the organic litter produced, containing little carbon and nitrogen, cannot support significant levels of denitrification. All of these studies, though conducted in extremely varied environments, support the fact that denitrification cannot take place if there is not enough nitrate to reduce. They also demonstrate that without a substantial energy source like organic carbon, microorganisms cannot reduce that nitrate through denitrification, regardless of the amount of nitrate present in the soil.

For this research, the effects of organic matter content in wetland soils on nitrous oxide production were studied in two significantly vegetated marshes and a newly constructed riparian wetland with little vegetation. The hypothesis is that the vegetated wetland basins should have higher concentrations of organic matter, a source of carbon, and therefore higher nitrous oxide production through denitrification than the unvegetated wetland basin.

Methods

Site description

Sediment samples and soil atmosphere samples were collected on occasions from mid-November 1997 through July 1998 from three wetlands at the Olentangy River Wetland Research Park (Fig. 1). Two of the wetlands are 1-ha marshes constructed in 1993 and the third, a 3-ha billabong, was constructed in 1996 as a mitigation project. One of the marshes (Wetland 1) was planted and the other (Wetland 2) left unplanted, but the coverage of vegetation was practically identical by 1997 (Mitsch et al., 1998). The billabong was planted in spring of 1997 but, as it has gone through only one growing season before the start of the research, the current degree of colonization by plants is insignificant in comparison to the two marshes. The marshes obtain river water through a water pumping system, while the billabong is fed by river water whenever the river rises above a certain level and by groundwater. All three wetlands also receive some water from precipitation.

Measurement for soil organic matter content

Twelve sites, four in each of the three wetlands, were sampled for organic matter content and nitrous oxide concentration (Fig. 1). In the marshes, two of these sites were near the respective inflows, along the basin margins, and two near the outflows, also along the basin margins. The billabong was sampled at two sites in the proximity of the

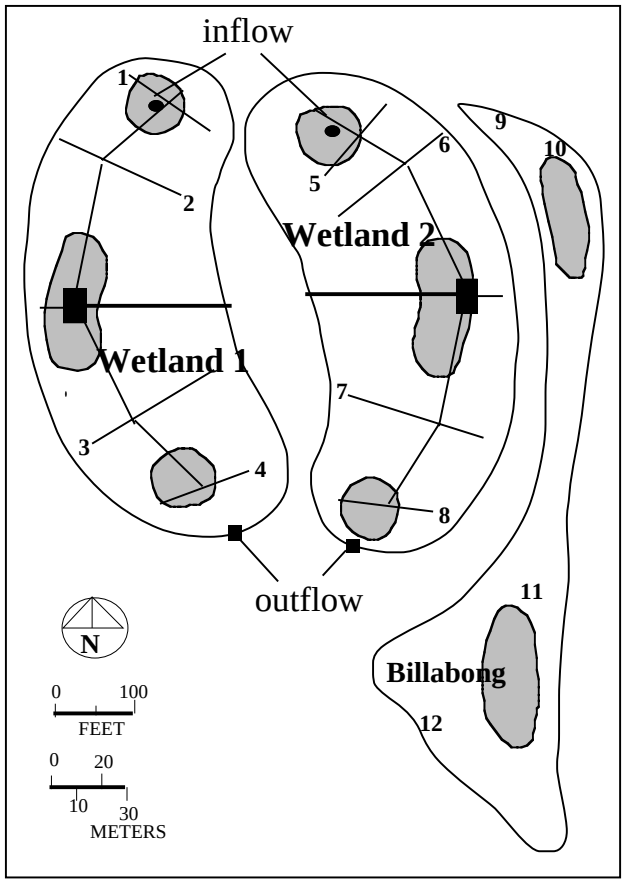

Figure 1. Location of the sampling sites for the denitrification study at the Olentangy River Wetland Research Park

inflow and two in the region at the southern end near the outflow. To obtain the organic matter content of the soil samples, cores 10 cm in length were extracted from just below the organic layer of the sediment. The cores were taken using a soil probe. The samples were placed in an oven to dry for 24 hours at 105° C. They were then weighed, placed in a muffle furnace for at least 12 hours at 500° C to burn off the organic matter, and weighed once more to determine the organic matter content. The organic matter content sampling was performed a total of three times over the course of the project, in November 1997 and in March and July 1998. These measurements took place in the Wetland Ecology Laboratory at The Ohio State University.

Measurement of nitrous oxide concentrations

In order to determine the nitrous oxide concentrations at each site, gas samples were withdrawn from sections of regular silicone tubing (12.7 cm long, 1.27 cm ID, 1.90 cm OD, 0.32 cm wall thickness, Cole-Palmer #E06411-55) that were left in the wetland soil for at least 8 hours. Both ends of the tubing were sealed with silicone caulking and allowed to dry for 48 hours before use in the field, as recommended by Jacinthe and Dick (1996). A syringe fitted with a 25-gauge needle, 3.81 cm in length, and a one-way stopcock was inserted into the silicone sealing to withdraw as much gas as possible from the tubing. The gas sample was then transferred to an evacuated 20-ml Wheaton serum bottle capped with a rubber stopper and an aluminum seal. Sampling was performed in the middle of each month of the project: November 1997 and March, April, May, June and July 1998. All samples were taken around noon for uniformity within the diurnal period.

Trace levels of nitrous oxide (N_2O), a product of denitrification, were measured with a Tremetrics Dimension I gas chromatograph equipped with an electron capture detector, Setra electronic manometer, and an integrator at the Soil Biochemistry and Microbiology Laboratory at the Ohio Agricultural Research and Development Center in Wooster, Ohio. A Precision Scientific vacuum pump was used to evacuate the serum bottles used in the field.

In order to calibrate the gas chromatograph (GC), known concentrations of nitrous oxide were prepared and fed into the GC. A field sample was run first to get an approximation of nitrous oxide concentration, followed by the nitrous oxide standards with concentrations around that of the field sample, to save time in the calibration. It also eliminated the need for higher concentration standards that could have flooded the system and taken hours to clear. The remaining field samples were then tested. A syringe was used to withdraw the sample from a serum bottle, the needle was removed, and the one-way stopcock was attached to an inert gas sampling valve on the GC apparatus. Once the manometer read zero to indicate that the system was evacuated, the stopcocks were flipped open and the GC was started. It took just less than 4 minutes for the nitrous oxide peak to occur, at which time the GC was automatically stopped. The manometer, which was increasing, steadied at the time of the peak and its pressure (psia) reading was recorded. Once the system cleared, the next sample was tested. The data printed out on the integrator.

Calculations

The weights of the soil core samples for each site, before and after the organic matter was burned off in the muffle furnace, were used to calculate the percent of organic matter in each sample, assuming that all of the it was removed in the furnace.

Nitrous oxide data were analyzed to determine the nitrous oxide concentrations of the soil atmosphere samples. A graph of gas peaks printed out on the integrator. The significant peak was around 3.48-3.49 minutes, which was the nitrous oxide peak. Also on the printout was a table of data that gave, among other things, the areas under the given peaks. The volume of the sample used in calculations was equal to the peak area for nitrous oxide divided by the psia (recorded from the manometer) at the time of the peak. Using the information for the standards, a regression curve was graphed of nitrous oxide concentration (ppm) vs. peak area/psia. Then nitrous oxide concentrations for the field samples were determined from the equation of the curve.

Results and discussion

Soil organic matter content

The average percent soil organic matter content for each of the three wetland basins over the entire study is displayed in Figure 2. The mean contents for Wetland 1 and the billabong were approximately 5.4 % with similar standard errors of 0.21 % and 0.23%, respectively. Wetland 2 had a higher overall average of approximately 6.3% organic matter content, with a standard error more than twice as large at 0.55%. This is because the range of values was largest for Wetland 2, from 4.4% to 12%. The difference between wetland average organic matter contents was not significant. Temporally, from November to June, the changes in soil organic matter content were just as insignificant in all three of the wetlands.

Table 1 presents the averages and standard errors for all three wetland groups in November, March, and June. In the soil samples for the billabong and Wetland 1 in November, the average percent organic matter content was found to be 6.0%. The content in Wetland 2 was higher (7.3%) because the sample for site 8 contained a large amount of organic matter (12%) (Table 2). The carbon content of the sampling sites ranged from 5.1% at site 12 to 12% at site 8. A t-test was performed to analyze the significance of the differences between the average contents of the three wetlands. With $\alpha = 0.05$ and ν (degrees of freedom) = 6, no significant difference was found between any of the values.

For the March samples from the billabong and Wetland 1, the average percent organic matter content was 5.1%. As in the November samples, the average content for Wetland 2 was higher than those for Wetland 1 and the billabong (Table 1). The average content for Wetland 2 was 6.0%.

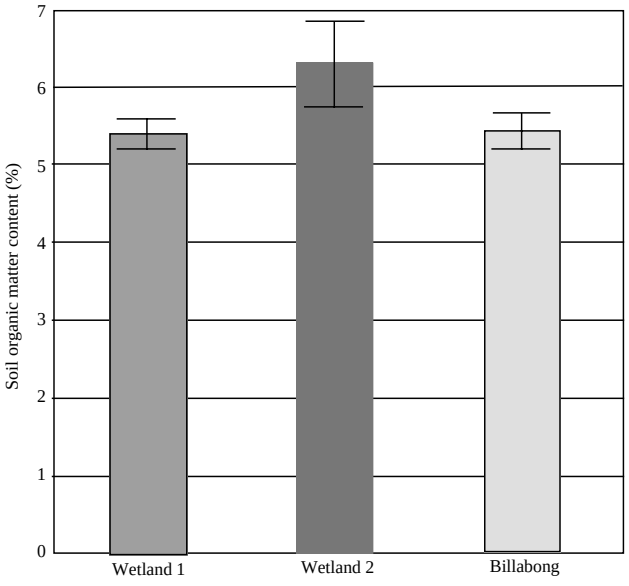

Figure 2. Average soil organic matter content (± standard error) for each wetland basin over the entire period from November 1997 to July 1998.

However, the standard error for the March Wetland 2 samples was much smaller than the standard error for the November samples. Once again, a t-test with $\alpha = 0.05$ and $\nu = 6$ showed no significant differences between the average contents for the wetlands.

The average percent organic matter content in June was 5.2% for Wetland 1, 5.6% for Wetland 2, and 5.1% for the billabong (Table 1). The differences between the wetlands are smaller for June than for either of the other two sampling months. Wetland 2 still had the highest average content, but not by much. T-tests with $\alpha = 0.05$ and $\nu = 6$ supported the

Table 1. Average (± standard error) soil organic matter content for each of the three wetlands for November 1997, March 1998, and June 1998.

Location	n	Soil Organic Matter (%)
November		
Wetland 1	4	6.0 ± 0.30
Wetland 2	4	7.3 ± 1.6
Billabong	4	6.0 ± 0.45
March		
Wetland 1	4	5.1 ± 0.30
Wetland 2	4	6.0 ± 0.35
Billabong	4	5.1 ± 0.25
June		
Wetland 1	4	5.2 ± 0.40
Wetland 2	4	5.6 ± 0.40
Billabong	4	5.1 ± 0.45

Table 2. Average (± standard error) nitrous oxide concentration for each of the three wetlands in November 1997 and March through July 1998.

Location	n	$N_2$0 Concentration (ppm)
November		
Wetland 1	4	8.48 ± 8.00
Wetland 2	4	0.280 ± 0.035
Billabong	4	202 ± 79.0
March		
Wetland 1	4	1.83 ± 0.385
Wetland 2	4	2.75 ± 0.630
Billabong	3	8.54 ± 2.94
April		
Wetland 1	4	1.86 ± 0.670
Wetland 2	4	1.39 ± 0.630
Billabong	4	4.33 ± 1.70
May		
Wetland 1	4	0.237 ± 0.0432
Wetland 2	4	0.319 ± 0.0670
Billabong	3	1.04 ± 0.293
June		
Wetland 1	4	0.974 ± 0.362
Wetland 2	4	2.42 ± 1.84
Billabong	4	48.5 ± 13.4
July		
Wetland 1	4	107 ± 95.0
Wetland 2	4	2.52 ± 0.660
Billabong	4	11.9 ± 2.50

conclusion that the differences between the three wetlands' organic matter contents were not significant.

Nitrous oxide concentrations

The seasonal pattern of the average N_2O concentrations for each wetland in the study period are shwon in Table 2. Mean concentrations for the billabong were often noticeably higher than those for Wetland 1 and Wetland 2. Only in July was the mean N_2O concentration for the billabong surpassed. In July, Wetland 1 had a much greater mean (107 ppm), but the standard error was very high (95.0 ppm), indicating a low confidence in the mean as a good estimate of the N_2O concentration in the wetland. In November, the standard error for the billabong was also very high. Other instances of relatively high standard error were Wetland 1 in November and the billabong in June. For Wetland 1, site 4 was responsible for creating the high error on both occasions. In the billabong, sites 11 and 12 yielded the high concentrations that resulted in the high standard errors. Overall, the ranges of concentrations for each site within a sample were moderately variable.

The average nitrous oxide concentrations for each of the three wetland basins over the entire study are presented in Figure 3. In this comparison of the basins, the differences between the wetlands are apparent. The billabong had the highest overall average at approximately 50 ppm N_2O. Wetland 1 had a mean concentration of 20 ppm for the period of the study, while Wetland 2 had the lowest mean concentration at 1.6 ppm N_2O. The highest standard error was found among the values for Wetland 1 (16 ppm): a few sample sites with high concentrations created a large deviation. Without these sites, the overall averages for Wetland 1 and Wetland 2 would have been much closer. Wetland 2 had the lowest standard error (0.38 ppm). The standard error for the billabong was approximately 4.7 ppm N_2O, a relatively low value for its high average. The billabong had consistently higher nitrous oxide concentrations than Wetland 1 and Wetland 2.

With $\alpha = 0.1$ and $\nu = 6$, a t-test showed that there was no significant difference between average nitrous oxide concentrations for Wetland 1 and 2 in November. However, nitrous oxide concentration differences were significant between the billabong and Wetland 1 and the billabong and Wetland 2. The mean concentration of N_2O in Wetland 1 was calculated to be 8.48 ppm. This mean did not reflect the individual values, however. Sites 1 through 3 ranged from 0.352 ppm to 0.593 ppm, but a value of 32.5 ppm was measured at site 4. The mean concentration for Wetland 2 was 0.280±0.035 ppm, a much lower mean and standard error than for Wetland 1. Results for the concentration calculations for the billabong were the most surprising, with a mean±std. error of 202±79 ppm. The two sites near the inflow were close, with 79.0 ppm N_2O at site 9 and 62.7 ppm at site 10. The two sites in the deeper end of the billabong, sites 11 and 12, had extremely high values of 386 ppm and 280 ppm, respectively.

T-tests ($\alpha = 0.10$) were performed to determine the significance of the differences between the average concentrations (Table 2) for the wetlands in March. The difference between Wetland 1 and Wetland 2 ($\alpha = 6$) was not significant. The mean concentration for Wetland 1 was calculated to be 1.83 ppm, and the mean for Wetland 2 was 2.75 ppm. However, the differences between Wetland 1 and the billabong ($\alpha = 5$) and Wetland 2 and the billabong ($\alpha = 5$) were significant. For the billabong, the mean concentration of N_2O was 8.54 ppm. The range of values was from 4.35 ppm at site 12 to 14.2 ppm at site 11, so the standard deviation was high. Site 9 was eliminated from the study for March because of a sampling error.

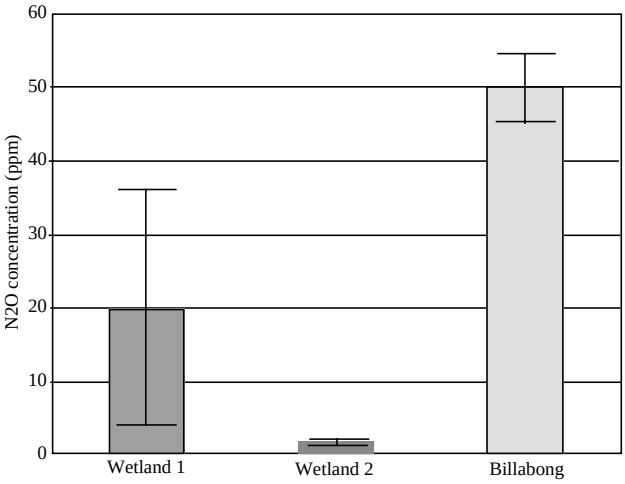

Figure 3. Average nitrous oxide concentrations (± standard error) for each wetland basin over the entire period from November 1997 to July 1998.

The mean concentration was higher in the billabong than in Wetland 1 or Wetland 2, but not to the extent that it was in November. In fact, it decreased significantly ($\alpha = 0.1$, $\nu = 5$), from 202 ppm to 8.54 ppm. At the same time, the average concentrations for Wetland 1 (minus November site 4) and Wetland 2 experienced significant increases ($\alpha = 0.1$, $\nu = 6$), from 0.467 ppm to 1.83 ppm and from 0.28 ppm to 2.75 ppm, respectively.

With $\alpha = 0.1$ and $\nu = 6$, t-tests showed that the differences between the mean N_2O concentrations for Wetland 1, Wetland 2, and the billabong were not significant in April, though the billabong continued to have the highest average concentration. These average concentrations are listed in Table 2. The greatest range was found in the billabong, from 1.01 ppm at site 12 to 8.59 ppm at site 10.

The mean concentration for Wetland 1 barely changed from March to April, increasing from 1.83 ppm to 1.86 ppm. The decreases that occurred from March to April in Wetland 2 and the billabong were also shown to be insignificant by t-tests, with $\alpha = 0.01$ and ν equal to 6 and 5, respectively.

The highest mean concentration among the three wetlands in May was in the billabong. The concentrations were very close in value, though. The average concentration was 0.237 ppm for Wetland 1, 0.319 ppm for Wetland 2, and

1.04 ppm for the billabong (Table 2). However, though the difference between Wetland 1 and Wetland 2 was insignificant ($\alpha = 0.1$, $\nu = 6$), the differences between Wetland 1 and the billabong ($\alpha = 0.1$, $\nu = 5$) and Wetland 2 and the billabong ($\alpha = 0.1$, $\nu = 5$) were significant. The fact that the lowest individual concentration for the billabong, 0.470 ppm, is higher than all but one (0.519 ppm at site 6) of the individual concentrations for the other two wetlands explains this. Site 11 was eliminated from the study because of an error in sampling.

From April to May, the concentration decrease in Wetland 2, from 1.39 ppm to 0.319 ppm, was insignificant ($\alpha = 0.1$, $\nu = 6$). The decrease in the billabong, from 4.33 ppm to 1.04 ppm, was also calculated to be insignificant ($\alpha = 0.1$, $\nu = 5$). For Wetland 1, the t-test indicated that the decrease was significant ($\alpha = 0.1$, $\nu = 6$), although the magnitude of change was not much greater than the change in Wetland 2. This is because the standard errors for Wetland 1 in April and May were very low, while those for Wetland 2 were relatively high.

The standard errors for the mean concentrations for each of the three wetlands in June are relatively high (Table 2). In Wetland 2, the standard deviation (3.68 ppm) was greater than the value of the mean (2.42 ppm). Site 6 had a concentration of 7.91 ppm, which was relatively high compared with the other 3 sites in Wetland 2 (0.387 ppm, 1.04 ppm, and 0.323 ppm). This explains the high deviation. In Wetland 1, the individual concentrations ranged from 0.310 ppm to 1.96 ppm, resulting in an average of 0.974 ppm with a standard error of 0.362 ppm. The billabong had the greatest range, from 22.2 ppm to 85.8 ppm, which explains the high standard error of 13.4 ppm. However, because the lowest individual concentration for the billabong was much greater than the highest concentration in either of the other two wetlands, there was a significant difference ($\alpha = 0.1$, $\nu = 6$) between the mean concentrations of Wetland 1 and the billabong and Wetland 2 and the billabong. The t-test analyzing the difference between the average concentrations of Wetland 1 and Wetland 2 found it to be insignificant ($\alpha = 0.1$, $\nu = 6$).

The average concentration in the billabong increased significantly ($\alpha = 0.1$, $\nu = 5$) from May to June, rising from 1.04 ppm to 48.5 ppm. The mean concentration increase in Wetland 2 from May to June was found to be insignificant ($\alpha = 0.1$, $\nu = 6$) because of the relatively high variability in individual concentrations. The increase in Wetland 1 was calculated to be significant ($\alpha = 0.1$, $\nu = 6$), once again, because of the smaller standard error values.

The range of individual concentrations for Wetland 1 in July was very large, from 0.246 ppm to 392 ppm. This resulted in an average of 107 ppm with a standard deviation almost twice that value at 190 ppm. This extremely large deviation led to t-test results for the differences between the mean concentrations of Wetland 1 and Wetland 2, and also Wetland 1 and the billabong, finding the differences to be insignificant ($\alpha = 0.1$, $\nu = 6$). A t-test performed for Wetland 2 and the billabong concluded that the difference between their average nitrous oxide concentrations, 2.52 ppm and 11.9 ppm (Table 2), respectively, was significant ($\alpha = 0.1$, $\nu = 6$). If site 4 (392 ppm) were excluded from the calculations, the mean for Wetland 1 would be much closer to those of Wetland 2 and the billabong.

The large standard error for Wetland 1 concentrations also explains why the change from June to July was calculated to be insignificant ($\alpha = 0.1$, $\nu = 6$). Site 3 (0.246 ppm) had a relatively low nitrous oxide concentration. If it were excluded, the increase from 0.974 ppm in June to July concentrations would have been significant. The mean concentrations for Wetland 2 in June and July were close, at 2.42 ppm and 2.52 ppm, respectively. The t-test with $\alpha = 0.1$ and $\nu = 6$ determined that the increase was insignificant. The decrease from June to July was significant ($\alpha = 0.1$, $\nu = 6$) for the billabong, though, falling from 48.5 ppm to 11.9 ppm.

Nitrous oxide concentration as a function of soil organic matter content

It was hypothesized that the data resulting from this experiment would show a significant difference between the soil concentrations of nitrous oxide for the unvegetated billabong and the vegetated marshes. The higher concentration of plants within the marshes would create a greater supply of organic carbon for the denitrification process because there is more litter for decomposition. The billabong, which has not had significant vegetation for an extended period of time, should have demonstrated nitrous oxide concentrations lower than those in the marshes.

The results of the testing demonstrated no obvious correlation between soil organic carbon content and nitrous oxide concentration (Fig. 4). Soil organic matter content did not vary a great deal among the sites, and changed very little

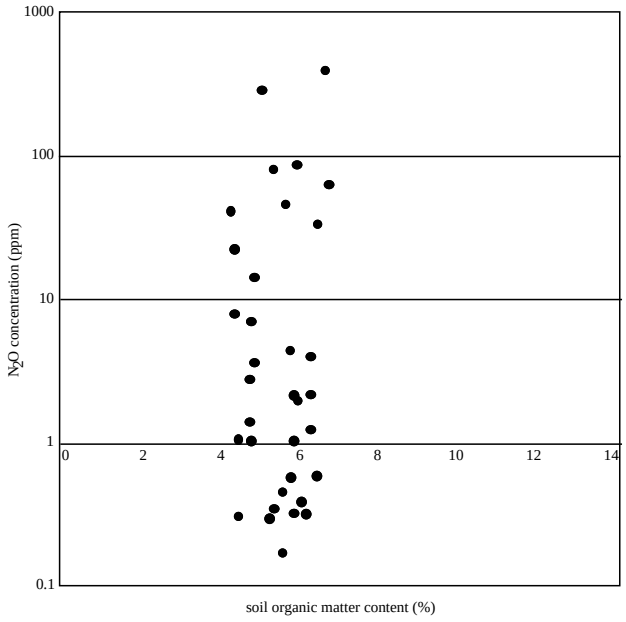

Figure 4. Nitrous oxide concentrations as a function of percent soil organic matter content for each site in November 1997 and in March and June 1998.

over the duration of the study. Therefore, the assumption that the lack of significant amounts of vegetation in the billabong would lead to lower levels of organic matter, and therefore organic carbon, in the soil than in the significantly vegetated marshes was false. It was also assumed that these low levels of organic carbon would produce relatively little nitrous oxide through denitrification because the energy source for the microorganisms would be comparatively insufficient. This assumption was also nullified, because the average nitrous oxide concentrations for the wetlands varied. From November to March, Wetland 1 and Wetland 2 experienced significant increases in mean concentration, while the mean for the billabong decreased significantly. The nitrous oxide concentrations for the billabong sites were much larger than those for Wetland 1 and 2 sites. The billabong experienced the most variation in mean N_2O concentration from month to month and from site to site, but the average organic matter content decreased less than 1% from November to June. The highest and lowest values for organic matter content did not correspond with those for nitrous oxide concentration. Therefore, the hypothesis of a direct relationship between the two variables cannot be supported with the data resulting from this research.

In a study conducted by Goodroad and Keeney (1984), it was observed that a drained marsh experienced much higher nitrous oxide levels than an undrained marsh. Accelerated decomposition of vegetation and mineralization of nitrogen in drained soils was given as an explanation for this observation. At the time of the sampling at the Olentangy River Wetlands, the billabong had no water above the ground surface, while both marshes had at least a few inches covering each site. This would indicate that nitrogen availability was a more significant limiting factor than carbon availability for the conditions present at the Olentangy River Wetlands in mid-November.

A more likely possibility for the higher denitrification potential measured in the new unplanted wetland (the billabong) compared to the 4-year-old experimental wetlands is related to the location of groundwater influence in the wetlands. The billabong is about 60 cm deeper in the ground than either of the experimental wetlands. It is more likely to be the recipient of groundwater flow (a discharge wetland) while the experimental basins are almost always perched above the water table and are thus recharge wetlands. The groundwater that the billabong intercepts is probably more reduced than the interstitial water in the experimental wetlands, which is recent river water. It appears that soil carbon is not as limiting to denitrification potential as are lower redox conditions in these basins.

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